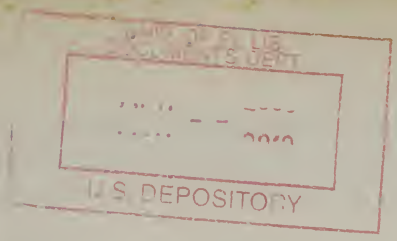

United States Department of Agriculture,

BUREAU OF CHEMISTRY—Circular No. 60.

H. W. WILEY, Chief of Bureau.

A THEORY OF THE INFLUENCE OF ACIDS AND ALKALIS ON THE ACTIVITY OF INVERTASE.

By C. S. Hudson, *Assistant Chemist.*

In alkaline solutions invertase shows no activity, in weakly acid solutions its enzymotic power reaches a maximum from which it decreases with increasing acidity. The simplest theoretical interpretation of this striking fact is that acids and alkalis combine with invertase by the principles of the law of mass-action and prevent it from inverting cane sugar. In the following calculations this hypothesis will be tested. If invertase combines with both acids and alkalis it is an amphoteric electrolyte and may be assumed to dissociate as follows:

(1) Invertase $\rightleftarrows \dot{H}$ + anion (acidic dissociation).

(2) Invertase $\rightleftarrows OH'$ + cation (basic dissociation).

If a units of invertase are dissolved in a unit volume of a solution containing hydrogen and hydroxyl ions in the fixed concentrations $(\dot{H})$ and (OH'), it will form x units of anion and y units of cation, leaving $a-x-y$ units of undissociated invertase. The mass-action law requires the fulfilment of the following conditions when equilibrium is attained:

$$(3)\ \frac{(x)\ (\dot{H})}{(a-x-y)} = K_1 \text{ and } (4)\ \frac{(y)\ (OH')}{(a-x-y)} = K_2$$

The quantity $a-x-y$ is the concentration of uncombined or undissociated invertase, and it is here assumed that the enzymotic activity is caused by this substance and is proportional to its concentration. Solving (3) and (4) for $a-x-y$ gives—

$$\text{Activity (i. e., } a-x-y) = \frac{a}{1+\frac{K_2(\dot{H})}{K_w}+\frac{K_1}{\dot{H}}} \qquad (5)$$

where K_w is the dissociation constant for water. This formula contains the three coefficients a, K_1, and K_2, which are of unknown

values; in order to determine the values of these coefficients the data which were found for the activity of invertase in three solutions containing small concentrations of hydrochloric acid are used, namely:[a]

HCl concentration. *Normal.*	Activity of invertase.
0.0005	62
.0015	61
.008	37

These data when introduced in equation (5) give the values $a=77$, $K_2/K_w=133$, and $K_1=0.000086$, and Formula 5 becomes—

$$\text{Activity}=\frac{77}{1+133(\dot{H})+\frac{0.000086}{(\dot{H})}} \qquad (6)$$

From this formula the activities of invertase over a considerable range of acidity and alkalinity have been calculated and are recorded in Table 1 in comparison with the activities which were found experimentally.

TABLE 1.—*Calculated and observed activities of invertase.*

HCl concentration.	Calculated activity.	Observed activity.
$(10)^{-9}$ (alkaline)	0.001	0.0
$(10)^{-7}$ (pure water)	.1	5.0
$(10)^{-6}$	.9	.....
$(10)^{-5}$	8.0	.....
0.00009	39	30
.0005	(62)	(62)
.001	63	63
.0015	(61)	(61)
.002	59	.....
.003	58	58
.005	45	.....
.008	(37)	(37)
.01	33	.....
.02	21	.....
.03	15	.....
.05	10	.....

By reference to the figure it will be seen that there is a remarkably close agreement between the calculated and observed activities. On account of the rapid destruction of invertase at 30° C. by acid above 0.01 normal the relation can not well be tested beyond that concentration, but it is probable that at 0° such measurements can be made, and this will be attempted in the near future.

The value of the quantity a, namely, 77, indicates that at no concentration of acid is all the invertase active, and at the point of maximum activity the proportion of invertase which is active is 63/77, or 82 per cent of all the invertase present.

[a] U. S. Dept. Agr., Bureau of Chemistry Cir. 55, p. 4.

The concentration of acid for which the enzymotic activity is a maximum can be calculated from Equation 5. Writing its first differential with respect to $(\dot{H})$ equal to zero and solving gives—

$$(\dot{H})_{max} = \sqrt{\frac{K_1 K_w}{K_2}} \qquad (7)$$

Substituting in this the values previously found for these constants gives $(\dot{H})_{max} = 0.8(10)^{-3}$ at 30° C. Sörensen,[a] from his experiments at 52°, has found this value to be approximately $(10)^{-4}$; our results are thus in fair agreement with his, but they can not be strictly

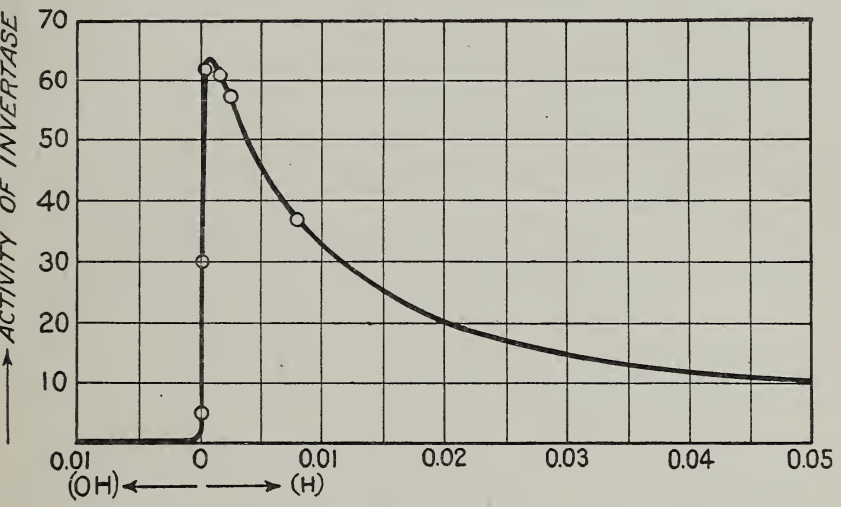

FIG. 1.—Graph of the formula for the activity of invertase.

compared because of the different temperatures used, namely, 52° and 32° C.

From the value of K_1 it is seen that invertase is a very weak acid, far weaker than acetic; from the value of K_2/K_w it is seen that invertase is a much weaker base, being only 133 times as strong as water itself.

[a] Comptes rendus des travaux du Laboratoire de Carlsberg, 1909, 8:132.